A FUNCTIONAL EXPLANATION FOR PATTERNS OF NORDITERPENOID ALKALOID LEVELS IN TALL LARKSPUR (*Delphinium barbeyi*)

M. H. RALPHS,* D. R. GARDNER, and J. A. PFISTER

USDA/ARS Poisonous Plant Laboratory
1150 E. 1400 N., Logan, Utah 84341

Abstract—Concentrations of norditerpenoid alkaloids vary among larkspur (*Delphinium*) species, locations, and years, but environmental stresses seem to have little effect on alkaloid levels. There is a need for a functional hypothesis of alkaloid synthesis and metabolism to explain the observed trends in concentration and to predict the toxicity of larkspur populations. This study was replicated at two locations over two years in the mountains of central Utah. Ten tall larkspur (*D. barbeyi*) plants were marked at each location, and a single stalk was harvested from each plant at weekly intervals throughout the growing season. Concentrations of toxic and total alkaloids were measured by Fourier-transformed infrared spectroscopy (FTIR), and alkaloid pools were calculated by multiplying the alkaloid concentration by the dry weight of the plant to determine the amount of alkaloids in the stalk. Alkaloid pools in the stalks increased for the first three weeks, leveled off, and then declined to low levels as the plants began to senesce. Concentrations of alkaloids declined through the season, as the alkaloids were diluted in the increasing biomass as the plants grew. These patterns will be used to predict potential toxicity of larkspur populations.

Key Words—Tall larkspur, *Delphinium barbeyi*, norditerpenoid alkaloids, methyllycaconitine, alkaloid pools.

INTRODUCTION

Tall larkspur (*Delphinium barbeyi*) is an important poisonous plant on mountain rangelands, causing acute poisoning and rapid death in cattle. There are many norditerpenoid alkaloids in the mountain species (Manners et al., 1993). Alka-

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*To whom correspondence should be addressed.

loids that contain the *N*-(methylsuccinimido)-anthranilic ester group (referred to as MSAL alkaloids) are the most toxic (Manners et al., 1995), with methyllycaconitine (MLA) being the prominent toxic one. We have separated the MSAL class from the other alkaloids in larkspurs and quantified them as a measure of toxicity (Gardner et al., 1997).

Total alkaloid concentrations are high in early growth and decline as the season progresses (Williams and Cronin, 1966). Concentrations of toxic alkaloids (MSAL group) also decline as the plants mature (Pfister et al., 1994; Ralphs et al., 1997). Alkaloid concentrations vary among species (Ralphs et al., 1997), locations, years (Ralphs et al., 1988), and individual plants (Ralphs et al., 1998a).

Environmental stresses did not alter toxic alkaloid levels. Short-term shade and photosynthesis inhibition decreased the dry weights of the plants, thus increasing the concentrations of alkaloids, but the total amounts of alkaloids remained constant (Ralphs et al., 1998a). Varying both light and temperature in a climate box study did not significantly affect alkaloid concentrations (Ralphs, unpublished data). The herbicide glyphosate (Roundup) rapidly desiccates larkspur plants, thus increasing alkaloid concentrations, but the amounts of alkaloids in the stalks were similar to untreated controls (Ralphs et al, 1998b). Treatment with metsulfuron (Escort) increased both concentrations and absolute amounts of alkaloids in tall larkspur. This has been the only treatment that has substantially increased the amount of alkaloids in larkspur. Insect damage from the larkspur mirid (*Hoplomachus affiguratus*), which is host-specific to tall larkspur, reduced toxic alkaloid concentrations (Ralphs et al., 1988c). Clipping tall larkspur greatly reduced both vigor and alkaloid pools the following year (Ralphs and Gardner, 2001).

There is need for a functional explanation of alkaloid synthesis and metabolism to explain the lack of effect from environmental stress, yet apparent variability between years, locations, and individual plants. Gershenzon (1994) presented a model of monoterpene synthesis in peppermint (*Mentha piperita*) that is consistent with our observations of diterpenoid alkaloids in tall larkspur. Enzymes required for biosynthesis of monoterpenes were present for only the first two weeks of growth. The total amounts of monoterpenes were produced during this short period and remained constant during the growing season, then were catabolized after the plants began to flower. Larkspur alkaloids appear to respond in a similar fashion.

The objective of this study was to test the hypothesis that alkaloids in tall larkspur are rapidly synthesized in the early, new growth stage of the annual growth cycle. After the first initial burst of growth, we predicted alkaloid synthesis would slow down or stop in relation to plant growth, leaving constant amounts of alkaloids for the remainder of the season. However, the concentrations of alkaloids should decline as the plants continue to grow and increase in biomass.

METHODS AND MATERIALS

The study was replicated at two locations in the mountains of central Utah. The Ferron Reservoir site was 46 km west of Ferron, Utah, at an elevation of 3150 m. It was in the subalpine zone, and the vegetation consisted of scattered subalpine fir stands interspersed in the tall forb plant community dominated by tall larkspur, western cone flower (*Rudbeckia occidentalis*), sweetcicely (*Osmorhiza occidentalis*), and mountain brome (*Bromus carinatus*). We fenced a 10 × 30 m exclosure to prevent any grazing interference during the experiment.

The second site, Mt. Terrell, was 40 km east of Salina, Utah, at 3230 m, in the ecotone of the aspen and subalpine zone. Tall larkspur dominated the tall forb community with violet (*Viola purpurea*), sedge (*Carex* spp.), and mountain brome as understory species. The site was on a steep side hill that was not grazed by large ungulates.

Both sites were snow drift areas that allowed access to the plants just as the snow melted and they started to grow. Ten uniform plants (30–40 stalks/plant) were randomly selected at each site in 1997 and marked with orange tent stakes with an aluminum number attached. One randomly selected stalk was harvested from each plant on a weekly basis from emergence in late June until senescence in September. The study was repeated on the same plants in 1998. The heights of the stalks were measured when harvested, then the stalks were placed in airtight plastic bags and immediately frozen with Dry Ice. Samples were freeze dried, weighed to obtain dry weights, then ground through a Wiley mill with a 1-mm screen. Alkaloids were extracted and concentrations of toxic and total alkaloids were measured by Fourier-transformed infrared spectroscopy (FTIR) (Gardner et al., 1997).

The quantity of toxic and total alkaloid pools in each stalk was calculated by multiplying the alkaloid concentration by the dry weight of the stalk. Alkaloid pools were used here to express the amount of alkaloids in a stalk, which better describes the dynamics than does alkaloid concentration. This is a better term than absolute amount of alkaloids that we used previously (Ralphs et al., 1998a–c). It is similar to the total nonstructural carbohydrate (TNC) pools used by Caldwell et al. (1981) to describe differences in photosynthesis, carbon storage, and growth between grass species. Bryant et al. (1993) used the term absolute dry mass of tannins per leaf to quantify increased synthesis of condensed tannins in Alaska paper birch, as a delayed response to insect herbivory the previous year. They concluded that concentration of secondary metabolites can be misleading because concentration changes if it is diluted in a larger pool or concentrated in a small pool. Ohnemis and Baldwin (1994) described finite nitrogen pools in wild tobacco and reported that defoliation simulating insect herbivory shifted allocation of nitrogen to nicotine synthesis at the expense of growth.

Data for toxic and total alkaloid concentration, toxic and total alkaloids pools, and stalk height and weight were analyzed by the mixed procedure analysis of variance in SAS, by using compound symmetry covariance in a split plot design. Location and year were the main effects, and plants within locations was the factor repeated over weeks of the split plot in time.

RESULTS

There was a difference in larkspur growth between years, as expressed in the stalk heights (Figure 1). Cooler temperatures in 1998 caused snow drifts at both locations to linger, thus delaying growth for one week at Ferron and two weeks at Salina (week × location × year interaction, $P = 0.001$). However, maximum stalk heights by late August were similar for both locations in both years ($P = 0.19$). To compensate for differences in the start of growth, alkaloid levels were compared at equivalent growing stages, not calender dates.

Concentrations of toxic and total alkaloids (Figure 2) were extremely high at the beginning of growth in 1997. These levels are the highest measured in tall larkspur (Ralphs et al., 1997). Williams and Cronin (1966) reported total alkaloid concentrations of 41 mg/g in Duncecap larkspur (*D. occidentale*) and 38 mg/kg in tall larkspur. Kreps (1969) reported total alkaloid concentrations in Duncecap larkspur of 50 mg/g.
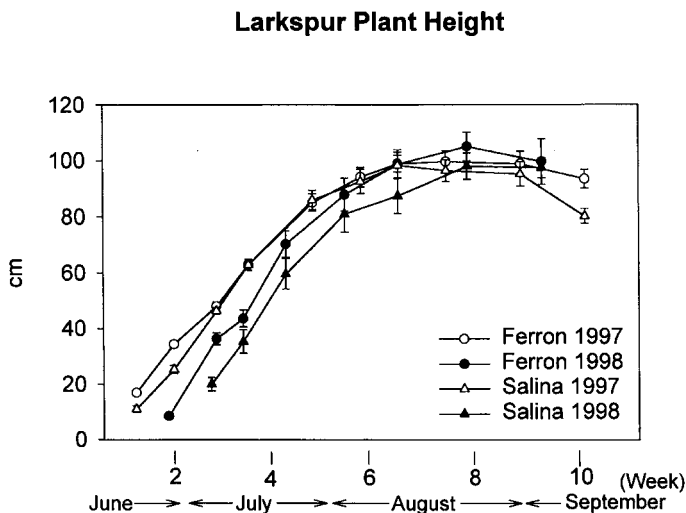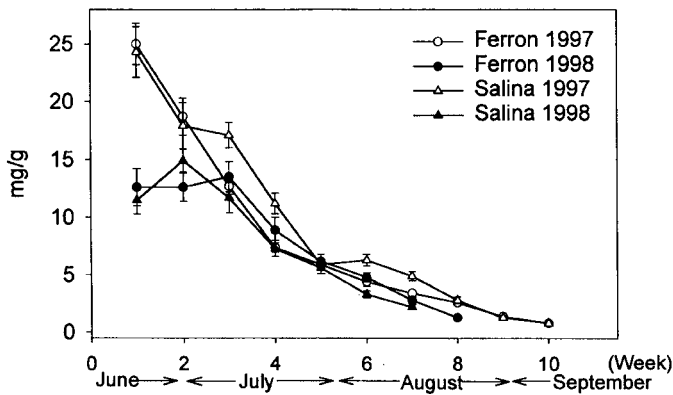
Larkspur Plant Height

FIG. 1. Larkspur plant height through the growing season.

Toxic Alkaloid Concentration
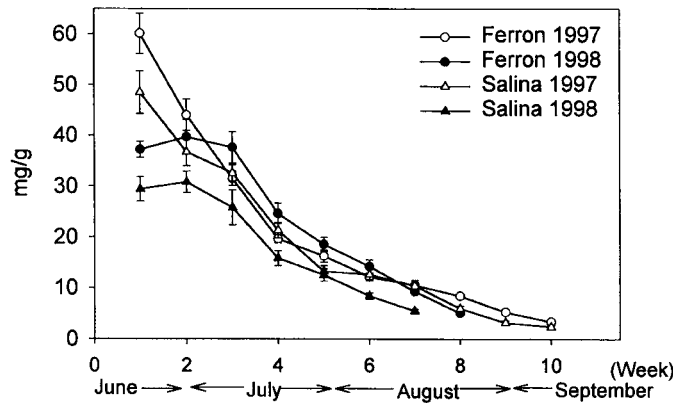

Total Alkaloid Concentration

FIG. 2. Concentrations of toxic and total alkaloids through the growing season.

Concentrations of both toxic and total alkaloids at the beginning of growth in 1998 were much lower ($P < 0.001$). This may have been due to cooler temperatures in 1998. Maximum June temperatures averaged 5°C lower than the 10-year average, and 4°C cooler than 1997. Environmental conditions during

initial synthesis may affect alkaloid concentration. However, after two weeks, concentrations of both toxic and total alkaloids were similar for both years and locations.

There were no differences in concentrations of toxic alkaloids among locations ($P = 0.79$). Total alkaloid concentrations were slightly higher ($P = 0.002$) at Ferron (Table 1). There were no differences in dry weights of larkspur stalks between locations or years ($P > 0.11$).

The alkaloid pools in larkspur stalks are the product of the alkaloid concentration multiplied by the dry weight of the stalk. There was no difference in pools of toxic alkaloids in larkspur stalks among locations ($P = 0.46$) (Table 1). However, there was a location $\times$ year interaction ($P = 0.05$); the pool was slightly lower at Salina in 1998.

The shapes of the curves of the toxic alkaloid pools were similar at both locations and in both years (Figure 3). The amounts of toxic alkaloids increased rapidly during the first three to four weeks then leveled off. This suggests that the alkaloids were synthesized in early growth, and then synthesis declined and stopped. If a constant amount of alkaloids remains in the plant as it continues to growth, the pool should remain constant over the remainder of the season. Instead, the pools of toxic alkaloids declined in the latter part of the growing season, reflecting possible catabolism or translocation to the roots. It is unlikely that the toxic alkaloids were metabolized to other alkaloids since the total alkaloid pools followed similar patterns (Figure 4).

DISCUSSION

Here we compare our results with some of the plant defense theories. Optimal plant defense theory (McKey, 1974; Feeny, 1976; Rhoades, and Cates, 1976) states that plants will allocate resources between growth, reproduction, and defense to optimize fitness. The optimal constitutive level of defenses balances metabolic costs of producing defense chemicals against the benefits in reduced loss of tissue to herbivory. Larkspur alkaloids appear to be constitutive rather than inductable. Alkaloid concentrations did not increase as damage occurred from the larkspur mirid, one of the few insects that feed upon larkspur (Ralphs et al., 1998c). However, the fact that few insects feeding on larkspur is an indication of the success of this defense strategy.

As part of the optimal plant defense theory, McKey (1974) suggested that toxic defense compounds should be concentrated in plant parts that most increase fitness of the plant. Alkaloid concentrations are highest in new growth of larkspur leaves, flowering racemes, and in seed pods (Ralphs et al., 1997; Pfister et al., 1994). Thus, even though the total pools are declining, these metabolic parts are protected to a greater degree that the rest of the plant.

TABLE 1. CONCENTRATION OF TOXIC AND TOTAL ALKALOIDS AND ALKALOID POOLS[a]

Alkaloids	Location	Toxic alkaloids			Total alkaloids		
		1997	1998	Mean	1997	1998	Mean
Alkaloid	Ferron	11.8 ± 1.0	8.7 ± 0.6	9.9 ± 0.6a	27.7 ± 2.2	25.5 ± 1.6	26.6 ± 1.4a
concentration	Salina	12.5 ± 1.0	8.2 ± 0.6	10.4 ± 0.6a	25.0 ± 1.8	18.6 ± 1.3	21.9 ± 1.2b
(mg/g)	Mean	11.8 ± 0.7a	8.5 ± 0.4b		26.4 ± 1.4a	22.1 ± 1.1b	
Alkaloid	Ferron	37.7 ± 1.9	39.4 ± 3.1	38.5 ± 1.8a	100.5 ± 5.0	118.4 ± 9.0	109.3 ± 5.2a
pools	Salina	38.6 ± 3.5	32.3 ± 1.4	35.5 ± 2.1a	79.4 ± 8.0	70.6 ± 4.8	75.0 ± 4.8b
(g/stalk)	Mean	38.1 ± 2.0a	35.9 ± 2.0b		90.0 ± 5.0a	94.5 ± 6.0a	

[a] Values are means ± SE. Means in columns for locations, or rows for years, followed by different letters differ significantly ($P < 0.05$).
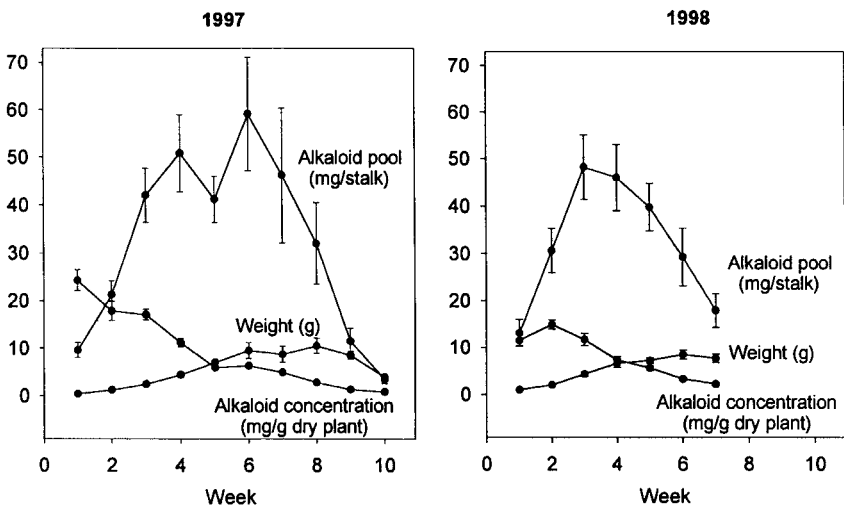
Toxic Alkaloid Pool
Ferron
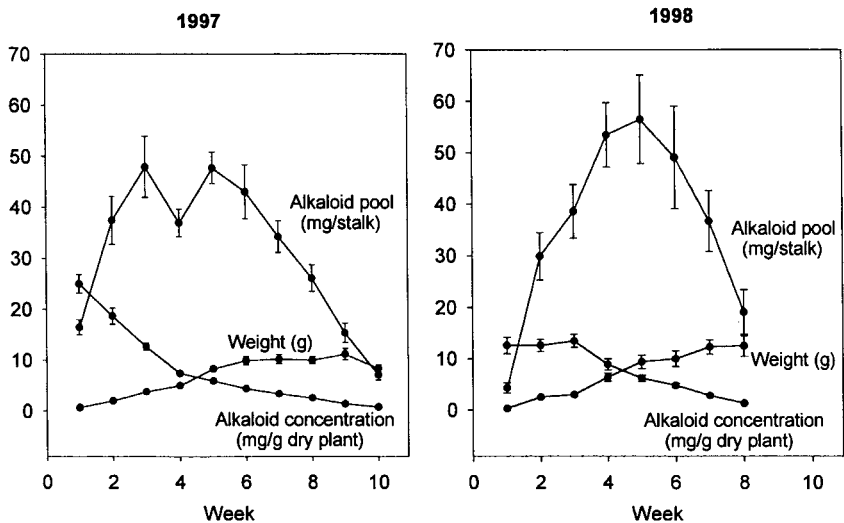
Salina

FIG. 3. Relationship between toxic alkaloid concentrations, dry weight of the stalk, and resulting pools of toxic alkaloids through the growing season.

Total Alkaloid Pool
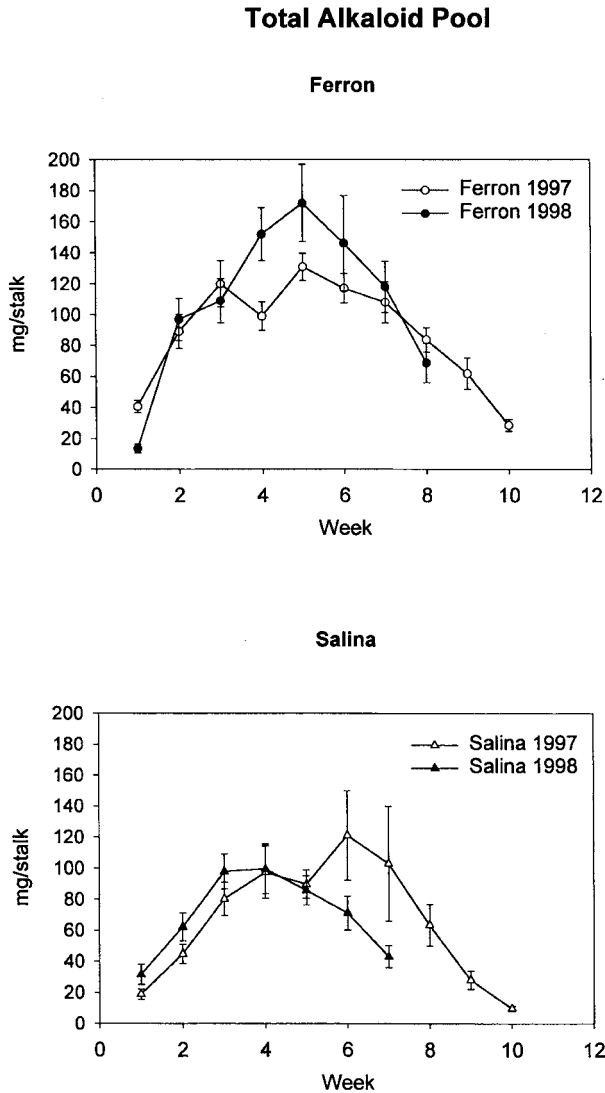
Ferron

Salina

FIG. 4. Total alkaloid pools through the growing season.

Resource availability theory encompasses an evolutionary time scale to explain the type of defenses that have evolved (Coley et al., 1985). In resource-rich habitats, competition favors rapidly growing plants with low levels of mobile N-based defenses. Tall larkspur grows in resource-rich habitats, exhibits

rapid growth rates, and its defense compounds are alkaloids derived from nitrogen early in the growing season. The alkaloids appear to be somewhat mobile in that they are rapidly synthesized early in growth, then are either translocated or catabolized toward the end of the growing season.

Carbon–nutrient balance theory functions on an ecological scale (Bryant et al., 1983) and suggests that the concentration of defense compounds are related to the current availability and the ratio of carbon to nitrogen. In resource-rich habitats, if growth is slowed, either by reduced photosynthesis or mild water stress (carbon limited), the available N is shifted to synthesis of N-based defense compounds. Previous research results (Ralphs et al., 1998a,b) showed larkspur alkaloids did not respond to short-term shade, photosynthesis inhibition, or desiccating herbicides; thus, larkspur apparently does not fit this theory.

Growth/differentiation balance theory (Herms and Mattson, 1992) states that early growth takes precedence over synthesis of defense compounds; then as growth slows and different parts and structures start to differentiate, resources are available for defense compounds. Our results are contrary to this theory, since alkaloid concentrations were highest in the new early growth, not later in the cell differentiation stages.

Finally, MSAL toxic alkaloids do not appear to be intermediate products in a biosynthetic pathway (Robinson, 1974), since they do not fluctuate rapidly in concentration and rate of turnover, but neither do they respond as typical end products that accumulate over the growing season (Harborne, 1991). Larkspur alkaloid concentrations were highest in early growth and declined over the season.

The amounts of both toxic and total norditerpenoid alkaloids in tall larkspur responded similarly to monoterpenes in peppermint (Gershenzon, 1994). The total amounts of monoterpenes in peppermint leaves increased during the first three weeks, then leveled out for several weeks during growth. However, during flowering, 50–70% of monoterpenes were catabolized to basic compounds that could be utilized for primary production. Enzymes for the synthesis of secondary compounds were present only in early growth of other plants, such as indole alkaloids in periwinkle (*Catharantus roseus*) (De Luca et al., 1988), and cyanogenic glycosides in sorghum (*Sorghum bicolor*) (Halkier and Moller, 1989). Other studies have reported early synthesis of secondary compounds, such as purine alkaloids from *Coffea arabica* (Frischknecht et al., 1986), alkaloids in *Cinchona* (Aerts et al., 1991), caffeine in tea leaves (Fujimori et al., 1991) and glucosinolates in rape leaves (Porter et al., 1986). Lerdau et al. (1994) cited several examples where monoterpene concentrations were highest in small expanding leaves.

Benn and May (1964) reported that diterpenoid alkaloids in *Delphinium brownii* are synthesized in roots and probably follow the mevalonate pathway, as do terpenes. However, a new pathway for terpenoid synthesis has been pro-

posed, starting from pyruvate and glyceraldehyde-3-phosphate (Lichtenthaler et al., 1997). If larkspur alkaloids are synthesized in roots, they may be translocated back to the roots at the end of the season. The large drop in the actual amounts of toxic and total alkaloids from their respective pools in the latter part of the growing season constitutes a substantial loss to the plant. The dry weight of a mature larkspur stalk is about 10 g. The total alkaloid pool ranged from a maximum of 120 mg/stalk at Salina to 180 mg/stalk at Ferron and dropped off to 20–30 mg/stalk. This represents a 1–1.6% decline in the dry weight of the stalk. Kreps (1969) reported that total alkaloid concentrations in Duncecap larkspur roots and crowns varied little during the growing season, except for increasing 0.5–1.0% as the new buds started to enlarge in August. It is possible that some, if not all alkaloids were translocated back to the roots in our study.

The major environmental factors that affect the physiological process of growth (light, photosynthesis, temperature, insects, and herbicides) did not affect alkaloid pools in mature tall larkspur plants in previous studies (Ralphs et al., 1998a–c). However, alkaloid concentrations during the first two weeks of 1998 were lower than in 1997. It is possible that the cold wet weather at the beginning of the 1998 season suppressed alkaloid synthesis. On the other hand, the lower alkaloid concentrations in 1998 may have been due to residual effects from harvesting stalks from the same plant the previous year, although less than one third to one half of the stalks were harvested at increasing stages of maturity. Perhaps carbohydrate storage was restricted in 1997, and there was not enough as the plants started to grow in 1998 to supply carbon or energy for alkaloid synthesis until new photosynthetic leaf material developed.

CONCLUSIONS

Alkaloids in tall larkspur appear to be synthesized during the first three to four weeks of early growth. Pools of alkaloids increase during this period, peak at four to six weeks, then decline as the alkaloids are apparently catabolized or translocated back to the roots. Concentrations of alkaloids steadily decline as the alkaloids are diluted in the increasing biomass.

REFERENCES

AERTS, R. J., VAN DER LEER, T., VAN DER HEIJDEN, R., and VERPOORTE, R. 1991. Developmental regulation of alkaloid production in Cinchona seedlings. *J. Plant Physiol.* 130:285.

BENN, M. H., and MAY, J. 1964. The biosynthesis of diterpenoid alkaloids. *Experientia* 29:252–253.

BRYANT, J. P., CHAPIN, F. S., and KLEIN, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–368.

BRYANT, J. P., REICHART, P. B., and CLAUSEN, T. P. 1993. Effects of mineral nutrition on delayed inducible resistance in Alaska paper birch. *Ecology* 74:2072–2084.

CALDWELL, M. M., RICHARDS, J. H., JOHNSON, D. A., NOWAK, R. S., and DZUREC, R. S. 1981. Coping with herbivory: Photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. *Oecologia* 50:14–24.

COLEY, P. D., BRYANT, J. P., and CHAPIN, F. S. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895–899.

DE LUCA, V., FERNANDEZ, J. A., CAMPBELL, D., and KURZ, W. G. W. 1988. Developmental regulation of enzymes of indole alkaloid biosynthesis in *Catharanthus roseus. Plant Physiol.* 86:447.

FEENY, P. 1976. Plant apparency and chemical defense. *Recent Adv. Phytochem.* 10:1–40.

FRISCHKNECHT, P. M., ULMER-DUFEK, J., and BAUMAN, T. W. 1986. Purine alkaloid formation in buds and developing leaflets of *Coffea arabica:* Expression of an optimal defense strategy? *Phytochemistry* 25:613–616.

FUJIMORI, N., SUZUKI, T., and HSHIHORA, H. 1991. Seasonal variation in biosynthetic capacity for synthesis of caffeine in tea leaves. *Phytochemistry* 30:2234–2248.

GARDNER, D. R., MANNERS, G. D., RALPHS, M. H., and PFISTER, J. A. 1997. Quantitative analysis of norditerpenoid alkaloids in larkspur (*Delphinium* spp.) by fourier transform infrared spectroscopy. *Phytochem. Anal.* 8:55–62.

GERSHENZON, J. 1994. The cost of plant chemical defense against herbivory: A biochemical perspective, pp. 105–173, *in* E. A. Bernays (ed.). Insect–Plant Interactions, Vol. V. CRC Press, Boca Raton, Florida.

HALKIER, B. A., and MOLLER, B. L. 1989. Biosynthesis of the cyanogenic glucoside dhurrin in seedlings of *Sorghum bicolor* (L.) Moench and partial purification of the enzyme system involved. *Plant Physiol.* 90:1552.

HARBORNE, J. B. 1991. The chemical basis of plant defense, pp. 45–59, *in* R. T. Palo and C. T. Robbins (eds.). Plant Defenses Against Mammalian Herbivory. CRC Press, Boca Raton, Florida.

HERMS, D. A., and MATTSON, W. J. 1992. The dilemma of plants: To grow or defend. *Q. Rev. Bot.* 67:283–335.

KREPS, L. B. 1969. The alkaloids of *Delphinium occidentale* S. Wat. PhD dissertation. Utah State University, Logan, Utah.

LERDAU, M., LITVAK, M., and MONSON, R. 1994. Plant chemical defense: Monoterpenes and the growth-differentiation balance hypothesis. *Trends Ecol. Evol.* 9:58–61.

LICHTENTHALER, H. K., ROHMER, M., and SCHWENDER, J. 1997. Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. *Physiol. Plant.* 101:643–652.

MANNERS, G. D., PANTER, K. E., RALPHS, M. H., PFISTER, J. A., OLSEN, J. D., and JAMES, L. F. 1993. Toxicity and chemical phenology of norditerpenoid alkaloids in the tall larkspurs (*Delphinium* species). *J. Agric. Food Chem.* 41:96–100.

MANNERS, G. D., PANTER, K. E., and PELLETIER, S. W. 1995. Structure–activity relationships of norditerpenoid alkaloids occurring in toxic larkspur (*Delphinium*) species. *J. Nat. Prod.* 58:863–869.

MCKEY, D. 1974. Adaptive patterns in alkaloid physiology. *Am. Nat.* 108:305–320.

OHNMEIS, T. E., and BALDWIN, I. T. 1994. The allometry of nitrogen allocation to growth and an inducible defense under nitrogen limited growth. *Ecology* 75:995–1002.

PFISTER, J. A., MANNERS, G. D., GARDNER, D. R., and RALPHS, M. H. 1994. Toxic alkaloid levels in tall larkspur (*Delphinium barbeyi*) in western Colorado. *J. Range Manage.* 47:355–358.

PORTER, D. A., and KIMMERER, T. W. 1986. Seasonal allocation of defense investment in *Ilex opaca* and constraints on a specialist leaf miner. *Oecologia* 69:217–224.

RALPHS, M. H., OLSEN, J. D., PFISTER, J. A., and MANNERS, G. D. 1988. Plant–animal interactions in larkspur poisoning in cattle. *J. Anim. Sci.* 66:2334–2342.

RALPHS, M. H., MANNERS, G. D., PFISTER, J. A., GARDNER, D. R., and JAMES, L. F. 1997. Toxic alkaloid concentration in tall larkspur species in the western US. *J. Range Manage.* 50:497–502.

RALPHS, M. H., MANNERS, G. D., and GARDNER, D. R. 1998a. Influence of light and photosynthesis on alkaloid concentration in larkspur. *J. Chem. Ecol.* 24:167–182.

RALPHS, M. H., MANNERS, G. D., and GARDNER, D. R. 1998b. Toxic alkaloid response to herbicides used to control tall larkspur. *Weed Sci.* 46:116–119.

RALPHS, M. H., GARDNER, D. R., JONES, W. A., and MANNERS, G. D. 1998c. Diterpenoid alkaloid concentration in tall larkspur plants damaged by the larkspur mirid. *J. Chem. Ecol.* 24:829–840.

RALPHS, M. H., and GARDNER, D. R. 2001. Influence at defoliation on toxic alkaloid concentration and alkaloid pools in tall larkspur. *J. Chem. Ecol.* (in review).

ROBINSON, T. 1974. Metabolism and function of alkaloids in plants. *Science* 184:430–435.

RHOADES, D. F., and CATES, R. G. 1976. Towards a general theory of plant antiherbivore chemistry. *Recent Adv. Phytochem.* 10:168–213.

WILLIAMS, M. C., and CRONIN, E. H. 1966. Five poisonous range weeds—when and why they are dangerous. *J. Range Manage.* 19:274–279.